Venous stasis and activation of coagulation

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Habilitationsschrift zur Erlangung der Venia Legendi

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Vorwort

Die vorliegende Habilitationsschrift umfasst Arbeiten, die im Verlauf meiner klinischen Forschungstätigkeit auf dem Gebiet der venösen Thrombose enstanden sind.

Die nachfolgend aufgeführten Originalartikel, ergänzt durch ein Editorial, werden als Habilitationsschrift eingereicht. Für das Verständnis des Themas wichtige Daten der Literatur werden im Sinne einer Synthese beigefügt und im Zusammenhang mit den eigenen Ergebnissen kommentiert.

Stricker H, Colucci G, Alberio L, Mombelli G. Variation in coagulation inhibitors during prolonged sitting: possible pathogenetic mechanisms for travel-associated thrombosis.
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Introduction

Virchow's triad which summarizes the pathogenetic mechanism of (venous) thrombosis includes alterations in blood flow (stasis), injury to the vascular endothelium, and alterations in the constitution of blood. The research of the past 150 years resulted in the description of the mechanisms of plasmatic coagulation, while, in the second half of last century, defects in the constitution of blood were identified as the cause of thrombophilia [1]. The mechanism by which blood stasis as one of the pathogenetic mechanisms can lead to the development of deep vein thrombosis even in healthy subjects, however, is incompletely understood. One situation associated with blood stasis in the lower limbs is a prolonged immoblisation in a sitting position, as is frequently encountered during a long (air) trip. The issues in the context of travel-induced thrombosis motivated the studies which will be presented hereafter.

Does venous stasis induce activation of coagulation?

When hemostasis is required, e.g. following a lesion in the vessel wall, thrombin is generated in a biphasic mode: During an initiation phase, picomolar amounts of the enzyme are formed due to the action of the complex TF-FVIIa on factor X. In the subsequent propagation phase, the majority of thrombin is formed due to the action of the intrinsic system [2]. To prevent excess thrombin generation, there are three partially interdependent major natural anticoagulant mechanisms which eventually result in a reduced thrombin formation or in an increased thrombin neutralization in vivo: the tissue factor inhibitory pathway which regulates the initiation phase, the heparin-antithrombin system, and the protein C pathway, which both control the propagation phase [3,4]. Also, in a so-called prethrombotic state which biochemically is defined by an increased level of prothrombin fragment 1+2 (F1+2, a marker of thrombin generation) but a normal level of fibrinopeptide A (FPA, a marker of fibrin formation) the coagulation is contained by natural inhibitors of thrombin [5]. Interestingly, low-grade thrombin generation has been shown to occur in healthy individuals both due to a minimal activation of

the extrinsic coagulation pathway [6] or in a tissue-factor independent manner [7]. In healthy individuals, however, the coagulation activation is maintained in a finely tuned equilibrium by the plasmatic inhibitors cited above.
If venous stasis is a risk factor for thrombosis, a prethrombotic state would be present and detectable by measuring the concentrations of the specific markers in a situation of compromised blood flow. In an experimental model, we imitated the situation during travel by immobilising healthy volunteers kept seated on a chair for 6 hours. At baseline, and after 3 and 6 hours, venous blood was drawn from the forearm in a manner as not to induce activation of coagulation. In 40 volunteers, we found no increase (which excludes coagulation activation) but a decrease in the concentration of F1+2 ($p=0.0001$), which is in line with a decreased thrombin generation during sitting. The decrease in F1+2 plasma level was both relevant in quantitative terms (-20% of the baseline value) and consistent across the volunteers (being present in 80% of them). FPA and D-dimer, the molecular markers of ongoing fibrin deposition and lysis, did not increase. These unexpected results contrasted with our original hypothesis and generated further questions: can the decrease in F1+2 concentrations be explained with circadian variations, or is it the consequence of venous stasis?

Issue of decreased thrombin generation while sitting

In order to disentangle the influence of a possible circadian variation from the effect of immobilisation we also examined a subgroup of the original volunteers during daily activity. Whereas during sitting the concentration of F1+2 decreased, we did not observe a change of this marker while the subjects were ambulant. In a second study, we applied tourniquets around the thighs of 10 volunteers and measured coagulation markers such as F1+2 at baseline and after 30 minutes in a blood sample drawn from a brachial vein. Again we found a significant decrease in F1+2 ($p=0.03$). Both findings resulting from two different models of immobilisation led to the conclusion that the decrease in thrombin generation was due to stasis and not to circadian variation. In the light of these results, the question remains if a decrease of

thrombin generation is protective or at the contrary a hazard for thromboembolism.

Possible mechanisms which link a decreased thrombin generation to a prethrombotic state: the protein C system

Thrombin has a dual function: as a procoagulant it is the key effector enzyme of the coagulation system, and as an anticoagulant it stimulates the expression of thrombomodulin and, by its binding to the latter, enhances protein C activation [1].

In a similar experimental model of subjects immobilised for 6 hours in a sitting position we assessed the protein C system by measuring the concentration of thrombomodulin and the activities of coagulation factor Va and VIIIa. At the end of the study, thrombomodulin decreased by 16% ($p=0.0001$), and the activities of both coagulation factors increased significantly ($p=0.04$ and 0.0004, respectively). These findings are consistent with an enhanced risk for coagulation activation: less thrombomodulin as well as higher activities of factor VIIIa were shown to correlate with a higher incidence of venous thrombosis [8, 9].

Venous thromboembolism after air travel was first recorded in 1954, but a relation between travel and thrombosis has been questioned until recently [10]. In a landmark study, however, the incidence of thromboembolism was shown to be related to air travel and depended on the flight duration [11]. An increase in the risk of thromboembolism in passengers undertaking long-haul flights may be caused by long term immobilisation in a cramped position, the cabin environment of hypobaric hypoxia, or dehydration, acting synergistically with personal risk factors. There has been inconsistent findings as to these mechanism that may induce coagulation activation during travelling. In a recent controlled study, hypobaric hypoxia did not result in coagulation activation, but the low number of included subjects could not exclude such an activation in more susceptible individuals [12].

Two recent studies addressing the issue of hypobaric hypoxia, both conducted within the framework of the WHO Research into the Global Hazards of Travel, provided contrasting results. The first study included

participants with no genetic thrombophilic risk factors. Individuals were exposed alternatively to hypobaric hypoxia (in a hypobaric chamber) and to normobaric normoxia. There was no diffference in parameters of coagulation, fibrinolysis, platelet function, and endothelial activation between the two experimental conditions [13]. In the second study it was shown that in susceptible subjects immobilised during an 8 hours flight, coagulation markers increased, which was not the case when immobilisation occurred for the same time period in a cinema, or during daily activities [14]. The conclusion of this study that a hypobaric hypoxic environment typically encountered in aircrafts is the main pathogenetic factor that explains travel-induced thrombosis, however, must be weighed against the fact that thrombosis can also result after a trip by bus or by train, a situation where prolonged sitting is not associated with hypobaric hypoxia. Our findings that thrombomodulin is downregulated by sitting suggest that venous stasis would induce a decrease in thrombomodulin expression, which in turn results in less activation of protein C and hence in less inactivation of coagulation factors Va and VIIIa. Support for this hypothesis comes from the study of Schreijer et al. where the subjects who developed the highest coagulation activation were more frequently carrier of the factor V Leiden mutation or took oral contraceptives, both conditions that negatively interfere with the performance of the protein C system [14]. Less thrombin in an intact vascular bed may thus confer a hazard, especially when other parameters negatively influence on the protein C system, and may be one of the mechanisms that link blood stasis to thrombosis.

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